Effect of the supplementation of a high concentrate diet with sunflower and fish oils on ruminal fermentation in sheep

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Abstract

This study was conducted to test the hypothesis that the supplementation of a high-concentrate diet with lipids, reportedly a good strategy for improving the nutritional value of ruminant-derived products, may not necessarily be associated with detrimental effects on ruminal fermentation in sheep. Four ruminally cannulated adult ewes were fed a high-concentrate diet, with no oil (Control diet), for a 14-day adaptation period. Afterwards, they were fed the same basal diet but supplemented with sunflower oil [20 g/kg fresh matter (FM)] and fish oil (10 g/kg FM) (SOFO diet) for a further 11 days, to investigate the impact of the addition of oils on the ruminal fermentation of the diet. On days 0 (Control), 3 and 10 of the experimental period rumen fluid was sampled at 0, 1.5, 3, 6 and 9 h after the morning feeding, for analysis of pH, and ammonia, lactate and total volatile fatty acid (VFA) concentrations. Alfalfa hay was incubated *in situ*, using the nylon bag technique, for 12 and 24 h to examine the effect of oil supplementation on ruminal disappearance of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF). On days 0 and 11, rumen fluid was collected just before the morning feeding and used to incubate alfalfa hay and the Control and SOFO diets by means of the *in vitro* gas production technique. The mean concentrations of acetate (87.8 *vs* 73.7 mmol/L) and butyrate (21.2 *vs* 17.7 mmol/L) were reduced by oil supplementation ($P< 0.05$) and the total VFA showed a tendency ($P= 0.098$) to be lower with the SOFO diet (139.0 *vs* 122.1 mmol/L). However, none of the other *in vivo* ruminal fermentation parameters were affected by the treatment ($P> 0.10$). The oil supplementation affected neither *in situ* rumen disappearance of DM, CP and NDF of alfalfa hay, nor rates of gas production ($P> 0.10$). On the other hand, a little, but significant reduction in cumulative gas production was observed when the experimental diets were incubated with rumen fluid derived from animals fed the oil-rich diet ($P< 0.05$).

Overall, the results suggest that the supplementation of high-concentrate diets with sunflower oil (20 g/kg FM) plus fish oil (10 g/kg FM) had little effect on ruminal fermentation and therefore its use to improve the nutritional value of ruminant-derived products cannot be precluded.

Keywords: lipid, rumen degradation, linoleic acid, ω-3 polyunsaturated fatty acids

Abbreviations: ADF, acid detergent fibre; AFR, average fermentation rate; CLA, conjugated linoleic acid; CP, crude protein; CPD, crude protein disappearance; DM, dry matter; DMD, dry matter disappearance; FA, fatty acids; FM, fresh matter; $LW^{0.75}$, metabolic weight; NDF, neutral detergent fibre; NDFD, neutral detergent fibre disappearance; OM, organic matter; PUFA, polyunsaturated fatty acids; SOFO, sunflower oil plus fish oil; VFA, volatile fatty acids

1. Introduction

Ruminant-derived products are the major source of conjugated linoleic acid (CLA) in the diet (Lawson et al., 2001). Feeding ruminants with vegetable oils rich in linoleic acid, such as sunflower oil, or fish oils, which are rich in ω-3 polyunsaturated fatty acids (PUFA), has proved to be an effective strategy for increasing CLA in milk (Shingfield et al., 2006). Benefits of some isomers of CLA, such as rumenic acid (*cis*-9, *trans*-11 C18:2), and ω-3 PUFA to human health (Williams, 2000) have motivated the current interest in lipid supplementation to ruminants.

Nevertheless, earlier studies on the addition of lipids to ruminant diets as an energy source raised concerns about detrimental effects of fatty acids (FA) on ruminal fermentation (Jenkins, 1993). Lipids are extensively hydrolysed in the rumen, rendering FA that have bacteriostatic and bacteriocidal effects. Among them, unsaturated FA are more antimicrobial than saturated ones (Harfoot and Hazlewood, 1997), and a differential toxicity of different PUFA to rumen microorganisms has also been observed (Maia et al., 2007). Dietary supplementation with oils has given inconsistent results on ruminal fermentation, with detrimental consequences (Fievez et al., 2003), no effects (Keady and Mayne, 1999; Beauchemin et al., 2007) and even positive responses (Sinclair et al., 2005). Controversial results may be due not only to the type (Wachira et al., 2000; Fievez et al., 2003) and amount (Doreau and Chilliard, 1997; Shingfield et al., 2008) of oil, but also to the basal diet composition. Palmquist and Griinari (2006) observed in cattle that a high-forage diet supplemented with a combination of sunflower oil and fish oil implied no adverse effects on rumen fermentation. However, Ueda et al. (2003) indicated that cattle fed high-concentrate diets were more prone to the detrimental effects of unsaturated FA, although our previous studies in lactating sheep (e.g., Gómez-Cortés et al., 2008) suggested that the supplementation of a concentrate-

rich diet with 6 g/kg dry matter (DM) of soybean oil did not affect *in vitro* ruminal fermentation.

On the basis of the latter studies, we hypothesized that the supplementation of a high-concentrate diet with lipids is not necessarily associated with detrimental effects on ruminal fermentation in sheep. The aim of this study was therefore to investigate the impact of the supplementation of a high-concentrate diet with a combination of sunflower oil (20 g/kg), rich in linoleic acid, and fish oil (10 g/kg), rich in ω-3 PUFA, on ruminal fermentation in ewes.

2. Material and methods

2.1. Animals and diets

Four individually penned Merino ewes (mean live weight 61.3 ± 10.80 kg), fitted with a ruminal cannula (40 mm internal diameter), were used. The diets, prepared in a feed mixer every week, consisted of a total mixed ration, based on alfalfa hay (particle size > 4 cm) and concentrate (forage:concentrate ratio 20:80) supplemented with 0 (Control diet) or 20 g of sunflower oil plus 10 g of fish oil/kg fresh matter (FM) (SOFO diet), whose ingredients and chemical composition are given in Table 1. Clean water and vitamin-mineral supplement were always available.

The experiment was performed in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes.

2.2. Experimental design

All the animals were offered the experimental diets (37 g DM/kg $LW^{0.75}$ and day) in two meals (60% at 9:00 h and 40% at 18:00 h) at 0.8 times the voluntary feed intake previously determined *ad libitum*, over two consecutive periods. First, the

Control diet was supplied for a 14-day adaptation period, and afterwards the animals were switched to the SOFO diet for 11 more days. *In vivo, in situ* and *in vitro* assays were conducted immediately before starting the administration of the SOFO diet, when the ewes were adapted to the consumption of the basal diet (day 0, Control), and after 3 days ($SOFO_3$; to study a possible prompt response) and 10 days ($SOFO_{10}$) of the administration of the SOFO diet. Even though the treatment might be confounded with the experimental period, this approach was used rather than a switch-back design in order to allow the adaptation of the sheep to the basal diet (i.e., the diet without supplementation) and to avoid a carry over effect of the oils on the rumen microbiota known to influence the ruminal fermentation (Wasowska et al., 2006). Similar experimental designs have been previously used for similar studies (e.g., Shingfield et al., 2003).

2.3. Experimental procedures

In vivo *studies*

Feed intake was monitored daily by weighing and drying the refusals. After 0 (Control), 3 ($SOFO_3$) and 10 ($SOFO_{10}$) days of sunflower plus fish oil supply, rumen fluid samples were collected via the cannula from each sheep at 0, 1.5, 3, 6 and 9 h after the morning feeding. Rumen fluid was strained through two layers of muslin, pH was measured and 4 mL were acidified with 4 mL of 0.2 M HCl for ammonia determination. Further aliquots of 4 and 0.8 mL of ruminal fluid were taken respectively for lactic acid and volatile fatty acid (VFA; deproteinized with 0.5 mL of 20 g/L metaphosphoric and 4 g/L crotonic acids in 0.5 M HCl) determinations. All samples were stored at –30 ºC until analysis.

In situ *studies*

With the same frequency, i.e., after 0 (Control), 3 ($SOFO_3$) and 10 ($SOFO_{10}$) days of SOFO supply, *in situ* rumen degradation of a reference feedstuff, alfalfa hay, was estimated. Nylon bags (150 × 100 mm; Ankom®, Macedon, USA) made of filter cloth with an approximate pore size of 50 µm of diameter were filled with 4 g of alfalfa hay [902 g organic matter (OM)/kg DM; 173 g crude protein (CP)/kg DM; 419 g neutral-detergent fibre (NDF)/kg DM], previously ground through a 2 mm screen. Bags were incubated in duplicate for 12 and 24 h (2 bags/time, 4 bags in total per animal), by suspending them in the rumen of each ewe just before the morning feeding. After removal from the rumen, the bags were washed with cold tap water and frozen (-30ºC) to help remove microbial attachment to feed particles. Once defrosted, bags were washed again with cold water in a commercial washing machine and dried in a forced-air oven at 60ºC to constant weight to determine DM disappearance (DMD). Concentrations of CP and NDF in the residues were measured to determine CP and NDF disappearances, respectively (CPD and NDFD).

In vitro *studies*

After 0 (Control) and 11 ($SOFO_{11}$) days of SOFO supply, *in vitro* ruminal fermentation was assessed using a modification of the gas production technique described by Theodorou et al. (1994), as adapted by Mauricio et al. (1999). Two rumen inocula were obtained from the ewes (2+2), pre-feeding, through the ruminal cannula. Rumen fluid was collected and transferred to the laboratory in pre-warmed thermos flasks and then strained through a double layer of muslin and kept under CO_2 flushing. The incubated substrates were alfalfa hay and the two experimental diets (Control and SOFO diets). Six samples per substrate (500 mg) [2 inocula (replicates) × 3 flask/inoculum] were incubated in sealed serum flasks at 39ºC with 10 mL rumen fluid and 40 mL phosphate-bicarbonate buffer. Buffer solution was prepared as described by

Goering and Van Soest (1970), with the exception that no trypticase was added, and pH was adjusted to 6.5 with orthophosphoric acid. Accumulated head-space gas pressures were measured with a pressure transducer at 2, 4, 6, 8, 10, 15, 18, 21, 24, 30, 36, 48, 72 and 96 h post-inoculation. Pressure values, corrected for the quantity of substrate OM incubated and gas released from blanks (i.e., rumen fluid plus buffer medium, without substrate; 3 flasks/inoculum), were used to generate gas volume estimates using a predictive equation derived from earlier simultaneous pressure and volume measurements (Hervás et al., 2005).

2.4. Chemical analyses

Experimental diets and *in situ* incubation residues were analysed for DM (ISO 6496:1999), ash (ISO 5984:2002) and CP (ISO 5983-2:2005). NDF and acid (ADF) detergent fibre were determined by the methods described by Mertens (2002) and the AOAC (2006; Official Method 973.18), respectively, using the Ankom technology (Ankom®, Macedon, USA). NDF was assayed with sodium sulphite and alpha amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by the filter bag technology (AOCS, 2008; Official Procedure Am 5-04), using an Ankom XT15 extraction system (Ankom®, Macedon, USA). Ammonia and lactic acid concentrations were determined by colorimetric methods (Weatherburn, 1967, and Taylor, 1996; respectively) and VFA by gas chromatography, with crotonic acid as internal standard (Ottenstein and Bartley, 1971), in centrifuged samples.

2.5. Calculations and statistical analysis

Gas production (G) data were fitted with time to the exponential model:

$$G = A \times (1 - e^{-c \times t})$$

where *A* represents the asymptotic gas production (mL/g OM), *c* is the fractional degradation rate (/h), and *t* is the gas reading time (h). Parameters *A* and *c* were estimated by an iterative least squares procedure using the procedure NLIN of the Statistical Analysis System package (SAS, 2003).

The average fermentation rate (AFR; mL gas/h) was defined as the average gas production rate between the start of the incubation and the time when the cumulative gas production was half of its asymptotic value, and was calculated according to the equation proposed by France et al. (2000):

$$AFR = A \times c / (2 \times \ln 2)$$

All data were analysed by one-way analysis of variance, using the MIXED procedure of the SAS (2003). The statistical model included the fixed effect of treatment (T; Control, $SOFO_3$ and $SOFO_{10}$ in the *in vivo* and the *in situ* data, and Control *vs* $SOFO_{11}$ in the *in vitro* gas production parameters). *In vivo* data taken over time (hours post-feeding) were analysed by repeated measures, using the MIXED procedure of the SAS (2003). The statistical model included the fixed effects of treatment (T), hours post-feeding (H) and their interaction. Means were separated using the 'pdiff' option of the 'lsmeans' statement of the MIXED procedure. Significant differences were declared at $P< 0.05$, and tendencies at $P< 0.10$.

3. Results

3.1. In vivo *studies*

The daily feed intake was not significantly affected by the experimental treatment and averaged 36 ± 0.3 g DM/kg $LW^{0.75}$. Rumen pH, and ammonia, lactate and VFA concentrations are presented in Table 2. Supplementation with SOFO had no effect ($P> 0.10$) on any of these ruminal fermentation parameters when analysed

separately for each time post-feeding, except for a lower acetate concentration ($P< 0.05$) observed at 3 h post-feeding in $SOFO_3$ sampling (i.e., after 3 days of SOFO supplementation). However, mean values of acetate and butyrate concentrations were significantly lower when animals were fed the SOFO diet ($P<0.05$), and the mean total VFA concentration showed a statistical tendency to be also lower with the oil supplementation ($P= 0.098$). The interaction between the treatment and the hours post-feeding (T x H) was never significant, and therefore is not included in the table. The acetate:propionate ratio (data not shown) was not affected by the experimental treatment, the average value being 3.64 ± 0.139 ($P> 0.10$). Nor were there any changes in molar proportions of acetate, propionate, butyrate or other VFA (calculated as the sum of isobutyrate, isovalerate, valerate and caproate) with oil supplementation, with average values of 0.61, 0.20, 0.14 and 0.05 mol/mol, respectively (data not shown).

3.2. In situ *studies*

Disappearances of DM, CP and NDF of alfalfa hay in the rumen were not affected by the dietary supplementation with SOFO, giving mean values of 0.729, 0.904, and 0.452 g/g incubated**,** respectively after 24 h of incubation (Table 3). After 12 h, approximately 0.90 of the DM and CP and 0.78 of the NDF that disappeared in the 24 h incubation period had been degraded.

3.3. In vitro *gas production*

In vitro gas production parameters are shown in Table 4. No significant differences between treatments were observed in the fractional degradation rate (*c*) and the average fermentation rate (AFR) for any of the incubated substrates. However, the incubations with inocula obtained after 11 days of SOFO supply ($SOFO_{11}$) showed a slight reduction in the cumulative gas production (*A*; $P< 0.05$) compared with those with

the inocula derived from animals fed the Control diet. This effect was not observed when alfalfa hay was incubated (P> 0.10).

4. Discussion

There is a widespread idea that PUFA can detrimentally affect ruminal fermentation. However, in the present study ewes were fed a high-concentrate diet supplemented with a combination of sunflower oil (20 g/kg) and fish oil (10 g/kg), and rumen fermentation was not significantly impaired, as it may have been expected from the low oil intake.

Lipids are hydrolyzed extensively in the rumen by microbial lipases, releasing long-chain fatty acids that may inhibit bacterial activity. Among long-chain FA, unsaturated ones are more antimicrobial than saturated ones (Harfoot and Hazlewood, 1997), biohydrogenation serving therefore to protect microbes from their toxic effect. Microbial toxicity of ω-3 PUFA, which are present in high amounts in fish oil, has been reported to be greater than the toxicity of linoleic acid (Maia et al., 2007), which is abundant in sunflower oil. However, in this study they seemed to exert no negative effects on the ruminal fermentation parameters studied *in vivo*.

First of all, the diet supplementation with oils had no effect on the feed consumption, contrarily to what was observed by Shingfield et al. (2006) when supplementing dairy cows with sunflower and fish oils. Nevertheless, the fact that the feed supply was fixed (at 0.80 times *ad libitum* intake, as above explained) precludes any conclusion on the lack of effect to be withdrawn.

Lactate was always present at normal concentrations (i.e., less than 5 mM; Owens et al., 1998), and the mean pH remained always within a physiological range (Krause and Oetzel, 2006). The pH was not affected by oil supplementation, in

agreement with previous *in vivo* studies using different lipid sources, including fish and sunflower oils (Fievez et al., 2003; Beauchemin et al., 2007). Shingfield et al. (2003) reported a higher pH when fish oil was included in the diet of cows, which was attributed to associated decreases in DM intake that were not observed in our study.

Ammonia concentrations were always greater than the 100 mg/L reported by Van Soest (1994) as optimal for the efficiency of amino acid synthesis and microbial growth, and were not affected by oil supplementation. Previous experiments with rumen fluid from sheep show inconsistent results, with significant increases or decreases in ammonia with linolenic or linoleic sources (Gómez-Cortés et al., 2008; Zhang et al., 2008). According to Shingfield et al. (2008), sunflower oil supplementation tends to reduce ammonia concentration in the rumen of cattle, whereas fish oil supplementation has been reported to increase it (Keady and Mayne, 1999). The lack of a significant effect on the concentrations of either ammonia or those VFA originating from the deamination of some amino acids (valerate and branched-chain VFA) would indicate that N metabolism was not altered in our study.

Although an inhibition of microbial activity by oil supplementation might lead to a reduction in total VFA concentration in the rumen, as Lee et al. (2005) observed with their highest level of fish oil inclusion, other studies involving sheep (Fievez et al., 2003) and cattle (Keady and Mayne, 1999; Shingfield et al., 2008) reported no significant effect of oil supplementation on total VFA concentration. In the current study, total VFA concentration was not affected at any specific time post-feeding, and only its mean value showed a trend ($P= 0.098$) to be lower with oil supplementation.

Concerning particular VFA, the addition of fish oil to the diet has often been reported to result in an increase in the molar proportion of propionate concentration and a decrease in acetate (Doreau and Chilliard, 1997; Keady and Mayne, 1999; Wachira et

al 2000; Fievez et al 2003). Similar results were observed when linoleic acid was incubated *in vitro* with rumen fluid from sheep (Zhang et al., 2008), whereas supplementation with sunflower oil in cattle did not affect the rumen VFA proportions (Beauchemin et al., 2007). The propionate concentration was not significantly changed in this study, whereas the average acetate concentration showed a reduction with oil supplementation. This suggests that acetate-producing bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, which are considered to be predominant cellulolytic bacteria in the rumen, may have been more inhibited by PUFA (Maia et al., 2007; Zhang et al., 2008).

From a physiological point of view, a shift in the rumen microbial communities may result in changes in biohydrogenation and, consequently, in the milk FA profile (Palmquist et al., 2005). Furthermore, a decrease in acetate concentration might contribute to a reduction in mammary *de novo* fatty acid synthesis, which requires acetate as a precursor (Doreau and Chilliard, 1997).

Regarding butyrate, the effect of oil supplementation is very inconsistent, with reductions with fish oil or linoleic acid (Fievez et al., 2003; Zhang et al., 2008), no effects with linoleic-rich sources or fish oil (Keady and Mayne, 1999; Beauchemin et al., 2007; Shingfield et al., 2008) and even increases with a combination of sunflower oil and fish oil (Palmquist and Griinari, 2006) having been reported. In the current study, the reduction in the mean butyrate concentration might be explained by variations in butyrate-producing bacteria, such as *Eubacterium ruminantium* and *Butyrivibrio fibrisolvens*. The former bacteria is known to be inhibited by PUFA (Maia et al., 2007), whereas the numbers of the latter species, which are tolerant to high concentrations of unsaturated fatty acids (Maia et al., 2007), are usually reduced in animals receiving high-concentrate diets (Harfoot and Hazlewood, 1997).

Ruminal degradation of DM, CP and NDF was not affected by oil supplementation in our study, in agreement with Keady and Mayne (1999), who observed no effect of fish oil supplementation, even when a shift in the rumen fermentation pattern was observed. The consequences of oil supplementation reported elsewhere, however, include reductions, no effects or even increases in fibre degradation (Wachira et al., 2000; Sinclair et al., 2005). In the present study, although a high-concentrate diet could have limited the number of predominant cellulolytic bacteria, which are usually more affected by oil supplementation (Doreau and Chilliard, 1997), other bacteria, able to degrade fibre, might have occupied their niches, which would explain the absence of any effect on the degradation of fibre.

In a previous study in milking ewes supplemented with sunflower and fish oils (results to be published elsewhere), significant changes in milk composition and fatty acid profile after only 3 days of lipid inclusion suggested a prompt response of the rumen microbiota. However, this hypothetic prompt modification of the microbial communities could not be confirmed in the current experiment because it was not reflected in remarkable differences between the two samplings carried out during the SOFO treatment ($SOFO_3$ and $SOFO_{10}$) on the studied ruminal fermentation parameters.

The effect of lipid supplementation on ruminal fermentation relies mainly on three factors, as previously mentioned: first, the type of oil (Wachira et al., 2000), second, the level of oil inclusion in the diet (Shingfield et al., 2008) and third, the dietary forage:concentrate ratio, since animals fed a concentrate-rich diet might be more likely to be adversely affected by oil supplementation (Ueda et al., 2003). In lactating cows, a high-forage diet supplemented with a combination of sunflower and fish oils only induced a transient reduction in total VFA concentration at 2 h post-feeding, but no effect was observed at 6 h (Palmquist and Griinari, 2006). In our study, although the

ewes were fed a high-concentrate diet, the dietary value of physically effective fibre (Mertens, 1997), with the concentrate including whole cereal grains and the alfalfa hay being supplied with a particle size greater than 4 cm, may explain the lack of a negative effect of oil supplementation on pH, ammonia, lactate and VFA concentrations, and *in situ* ruminal degradation of DM, CP and NDF, suggesting that the rumen function was probably adequate.

In vitro gas production was slightly, although significantly, reduced when inocula from the animals fed the oil-rich diet were used to incubate either the Control (-3.6 %) or the SOFO diet (-6.9 %). The inocula derived from the animals fed the SOFO diet induced a decrease in *in vitro* gas production when both experimental diets were used as substrates compared to the inocula from the animals fed the Control diet, probably due to changes in the rumen microbial communities produced by the oil supplementation, as previously thought. The fact that the batch cultures of rumen microorganisms are closed systems renders them especially reliable for the detection of small changes in fermentation, which might explain why little reductions in gas production were found statistically significant.

5. Conclusion

The supplementation of a high-concentrate diet with a combination of sunflower oil (20 g/kg) and fish oil (10 g/kg), reportedly a good strategy for increasing the nutritional quality of ruminant-derived products, did not seem to remarkably impair ruminal fermentation in ewes. However, a shift in the VFA profile was observed, with decreases in acetate and butyrate concentrations, probably due to variations in the rumen microbial composition. Nonetheless, rumen function seemed to be maintained with the oil-rich diet, suggesting that microorganisms can adapt rapidly to fat supplementation and degrade the diet normally. It is probably worth mentioning that

these results are based on a relatively small number of observations, due to the complexity of using cannulated animals, and further research involving longer-term experiments and molecular-based studies on rumen microbiota would be advisable.

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Table 1

Ingredients and chemical composition of the experimental diets: Control diet (with no oil supplementation) and SOFO diet (supplemented with 20 g of sunflower oil plus 10 g of fish oil per kg of fresh matter).

	Diet	
	Control	SOFO
Ingredients (g/kg FM)		
Dehydrated alfalfa hay	200.0	194.0
Whole maize grain	250.0	242.5
Soybean meal	200.0	194.0
Whole barley grain	150.0	145.5
Beet pulp	90.0	87.2
Molasses	65.0	63.1
Salts[1]	40.0	38.8
Minerals and vitamins[2]	5.0	4.9
Sunflower oil[3]	0.0	20.0
Fish oil[4]	0.0	10.0
Composition (g/kg DM)		
Organic matter	890.8	886.6
Crude protein	184.7	191.5
Neutral detergent fibre	225.7	219.1
Acid detergent fibre	137.8	129.1
Ether extract	24.7	65.8

[1] Containing $NaHCO_3$ (375 g/kg), $CaCO_3$ (350 g/kg), Ca_2HPO_4 (150 g/kg) and mine salt (125 g/kg).

[2] INA OV1 (EVIALIS, Madrid, Spain).

[3] Sunflower oil contained (% total fatty acid methyl esters) C16:0 (7.5), C18:0 (4.3), C18:1 (26.3), and C18:2 (60.5).

[4] Fish oil (semi-refined tuna and sardine oil; AFAMPES 121 DHA, AFAMSA, Spain) contained (% total fatty acid methyl esters) C16:0 (21.4), C18:0 (5.9), C18:1 (14.9), C18:2 (1.7), C20:5 (6.3), and C22:6 (17.8).

Table 2

Post-feeding changes (at 0, 1.5, 3, 6 and 9 h after the morning feeding) in pH, and ammonia (mg/L), volatile fatty acid (VFA; mmol/L) and lactic acid (mmol/L) concentrations in the rumen liquid of animals on treatments Control, $SOFO_3$ and $SOFO_{10}$.

		Treatment[1]			sed	Significance[2] (P)	
	Time (h)	Control	$SOFO_3$	$SOFO_{10}$		T	H
pH	0	6.64	6.74	6.80	0.284	0.860	
	1.5	6.16	6.34	6.32	0.141	0.412	
	3	6.07	6.18	6.13	0.182	0.836	
	6	6.19	6.24	6.22	0.240	0.980	
	9	6.37	6.41	6.64	0.220	0.460	
	Mean	6.28	6.38	6.42	0.069	0.382	<0.001
Ammonia	0	238.7	354.5	310.0	55.93	0.167	
	1.5	342.0	378.9	362.2	49.76	0.765	
	3	312.9	290.1	360.9	61.61	0.527	
	6	205.8	200.0	258.8	47.41	0.428	
	9	193.4	272.7	233.0	49.02	0.317	
	Mean	258.5	299.3	305.0	16.50	0.108	<0.001
Total VFA	0	89.4	78.2	68.5	21.84	0.646	
	1.5	177.0	146.4	154.9	15.47	0.180	
	3	174.7	144.5	166.0	16.18	0.214	
	6	127.4	118.7	127.6	21.20	0.916	
	9	126.2	116.1	100.2	20.16	0.459	
	Mean	139.0	120.8	123.4	6.28	0.098	<0.001
Acetate	0	55.6	46.2	39.1	13.60	0.507	
	1.5	110.8	89.0	91.2	10.39	0.124	
	3	110.1[a]	88.1[b]	102.1[a]	6.17	0.018	
	6	81.7	73.0	76.6	12.85	0.796	
	9	81.0	71.2	60.4	12.00	0.282	
	Mean	87.8[a]	73.5[b]	73.9[b]	3.58	0.009	<0.001
Propionate	0	13.5	11.9	12.7	5.56	0.961	
	1.5	30.0	29.0	30.9	7.01	0.965	
	3	30.8	31.2	34.6	9.17	0.905	
	6	22.6	25.1	28.6	10.41	0.850	
	9	21.4	21.9	21.4	7.33	0.998	
	Mean	23.7	23.8	25.6	2.56	0.836	<0.001
Butyrate	0	14.6	13.7	11.5	3.47	0.668	
	1.5	28.1	21.2	24.5	3.45	0.184	
	3	26.6	18.8	22.2	3.92	0.194	
	6	18.0	15.9	17.4	2.84	0.749	
	9	18.8	17.3	13.8	3.14	0.312	
	Mean	21.2[a]	17.4[b]	17.9[b]	1.07	0.029	<0.001
Lactic acid	0	0.66	0.63	0.77	0.168	0.711	
	1.5	1.15	1.17	1.16	0.477	0.999	
	3	0.66	0.57	0.70	0.067	0.174	
	6	0.56	0.48	0.58	0.114	0.687	
	9	0.86	0.61	0.74	0.326	0.747	
	Mean	0.78	0.69	0.79	0.087	0.692	0.021

[1] Control = after 0, $SOFO_3$ = after 3, and $SOFO_{10}$ = after 10 days of sunflower and fish oil supplementation.

[2] T: treatment; H: hours post-feeding.

[a,b] Different superscripts indicate significant differences within a row ($P<0.05$).

Table 3

Disappearance (g/g incubated) of dry matter (DMD), crude protein (CPD) and neutral detergent fibre (NDFD) from alfalfa hay incubated for 12 and 24 h in the rumen of animals on treatments Control, $SOFO_3$ and $SOFO_{10}$.

		Treatment[1]			sed	Significanc
		Control	$SOFO_3$	$SOFO_{10}$		(P)
DMD	12 h	0.653	0.659	0.662	0.0254	0.943
	24 h	0.729	0.726	0.732	0.0209	0.965
CPD	12 h	0.828	0.847	0.843	0.0125	0.298
	24 h	0.900	0.908	0.905	0.0075	0.586
NDFD	12 h	0.341	0.358	0.361	0.0482	0.904
	24 h	0.452	0.441	0.464	0.0422	0.965

[1] Control = after 0, $SOFO_3$ = after 3, and $SOFO_{10}$ = after 10 days of sunflower and fish oil supplementation.

Table 4

In vitro gas production parameters (*A*, mL/g OM and *c*, /h) and average fermentation rate (AFR, mL/h) for each substrate (alfalfa hay, Control diet and SOFO diet) incubated with rumen inoculum derived from animals on treatments Control and $SOFO_{11}$.

	Treatment[1]		sed	Significance (P)
	Control	$SOFO_{11}$		
Cumulative gas production (*A*)				
Alfalfa hay	258.0	249.9	4.74	0.232
Control diet	346.8[a]	334.3[b]	2.64	0.041
SOFO diet	346.2[a]	322.2[b]	5.49	0.049
Fractional rate of gas production (*c*)				
Alfalfa hay	0.097	0.091	0.0084	0.528
Control diet	0.080	0.081	0.0019	0.632
SOFO diet	0.078	0.082	0.0057	0.533
Average fermentation rate (AFR)				
Alfalfa hay	18.02	16.33	1.212	0.297
Control diet	19.95	19.48	0.575	0.499
SOFO diet	19.33	19.00	1.102	0.790

[1] Control = after 0, and $SOFO_{11}$ = after 11 days of sunflower and fish oil supplementation.

[a,b] Different superscripts indicate significant differences within a row ($P<0.05$).